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Planktonic Larvae of Lamellibranch Mollusks from the Pontevedra Estuary, Northwest Spain: Methodology and Systematics*

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Key Words: Planktonic Larvae, Lamellibranch Mollusks, Methodology, Systematics, Pontevedra Estuary, Northwest Spain.

SUMMARY: Planktonic Larvae of Lamellibranch Mollusks from the Pontevedra Estuary, Northwest Spain: Methodology and Systematics. This paper describes the taxonomic groups of lamellibranch larvae identified in the plankton of the Pontevedra Estuary. The methods used in sampling, preparation, mounting and observation are also discussed.

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The quantitative validity of the samples has been considerably improved through the use of a sampling method consisting of filtering 20-litre water samples through 47 µm-mesh nylon filters. Scanning-electron micrography techniques (SEM) have been used to observe and identify the larvae, with a single mount for each complete sample, concentrated by filtration through 0.8 µm millipore filtres, which has streamlined the study considerably. Eight taxons have been identified, two at the species level (Mytilus edulis L and Cerastoderma edule (L.)); two at genus level (Venerupis sp. Lmk and Venus sp. L); three families (Pectinidae, Solenidae and Tellinidae); and last a group formed by Ostrea edulis L and Anomia ephipium (L), which have been considered together since their morphology does not permit a clear distinction to be made between them.

This paper forms part of the "Study of the Bionomic and Dynamic Impact of the Effluents of the Pontevedra Pulp and Paper Factory (Celulosas de Pontevedra)" being conducted at the Fisheries Research Institute of Vigo with the cooperation of the Fisheries Research Institute of Barcelona.

Introduction

Successful colonization of fixed substrata by betonic marine ogranisms depends, to a large extent, on the conditions under which their planktonic larval stages appear and on their development and transport.

In the case of lamellibranch mollusks in highly productive areas such as the Low Estuaries (Rias), control and rational exploitation of the populations requires extensive knowledge of their biology and although abundant bibliography exists with respect to their betonic stages (Figueras, 1956, 1957, 1960, 1966, 1979; Andreu, 1960, 1963; Figueras and Andreu, 1968; Aguirre, 1979; Perez-Camacho and Roman, 1979; Perez-Camacho, 1980, among others), hardly any data exist on their pelagic larval stages (Vives, 1960). The descriptions of the pelagic stages are based on examples cultured in the laboratory (Bernard, 1895, quoted by Le Pennec, 1980; Jorgensen, 1946; Rees, 1950; Loosanof and Davis, 1963; Loosanof et al., 1966; Chandley and Andrews, 1971; Pascual, 1971, 1972; Perez-Camacho and Roman, 1973; Perez-Camacho et al., 1977, etc.) and take into account both external morphology and the structure of the hinge and shell (colour, size, etc.) and different characteristics of their internal organization. With the use of scanning-electron micrography techniques (SEM), the details of the hinge structure have become of primary importance for identification (Le Pennec, 1974, 1980; Lutz and Hidu, 1979).

With regard to the systematics and seasional variations of lamellibranch larvae in natural plankton populations, all that is known is the paper by Brenko (1971) on Adriatic populations, and this points to an obvious gap that must be filled.

This paper describes the taxonomic groups of lamellibranch larvae identified in the Pontevedra Estuary, the criteria adopted for their classification, and the methods used both in sampling and in preparing, mounting and observing the larvae.

MATERIALS AND METHODS

Description of the zone studied and sampling strategy

The Pontevedra Estuary is located between the Arosa Estuary to the north and the Vigo Estuary to the south (Fig. 1), and its morphology and hydrographical characteristics are similar to those of the latter (Fraga, 1979). After the first exploratory campaign (12 February 1980) during which the spatial distribution of lamellibranch larvae was studied (Fig. 2), it was decided to follow the evolution over time of the populations at Station 1, using supplementary data from Stations 2 and 3 if necessary. Sampling was conducted once a month, under tidal conditions that were as similar as possible, from March 1980 to March 1981.

Sampling

The drawbacks of sampling plankton using nets in eutrophic zones (Alcaraz, 1977) increase in the case of lamellibranch larvae due to their small size (80-350 μm), which makes it necessary to reduce the mesh size used, leading to rapid clogging of the nets. Under these conditions, discrete sampling appears to be the most efficient way of obtaining reliable quantitative data and of gaining precise information

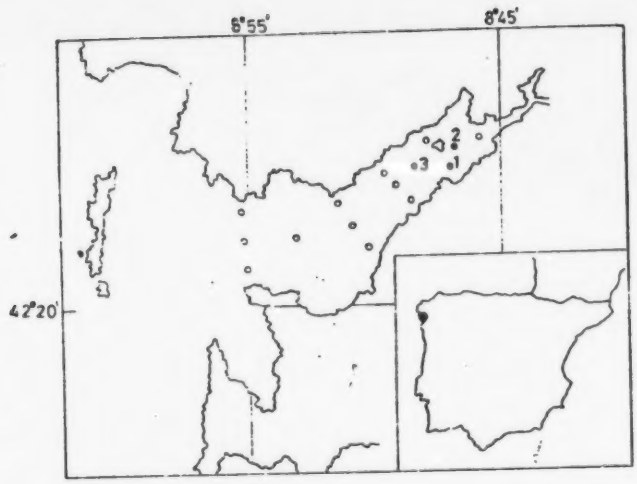

Fig. 1. Map of the Pontevedra Estuary showing the distribution of the Stations sampled in the preliminary study (12 February 1980); the samples used for this study come from Stations 1, 2 and 3 (black dots).

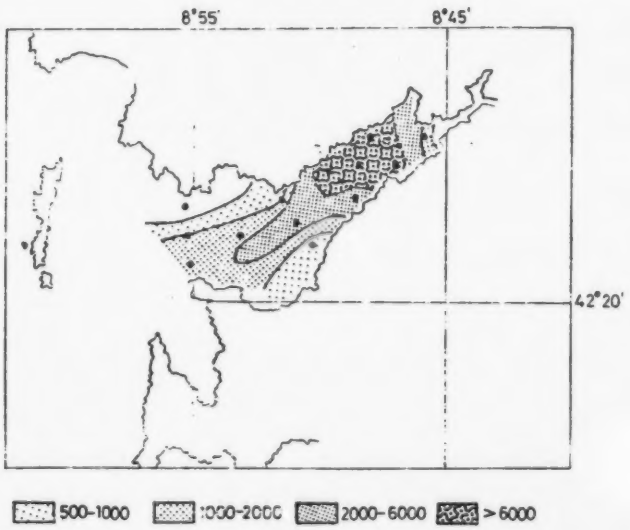

Fig. 2. Average distribution (ind. m^{-3}) of the total number of lamellibranch larvae in the Pontevedra Estuary during the intensive preliminary study (12 February 1980).

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on the sampling depth. It is also the only way of establishing statistical ratios between hydrographic variables and plankton populations in zones with highly heterogeneous environments.

The samples taken were taken using 20-litre capacity Van Dorn-type bottles made of transparent methacrylate (Alcaraz, 1977, 1979), at depths of 0.5 m and 10 m. The water was filtered by gravity through 47 µm mesh-size nylon filters and the material retained was fixed on board the vessel in 70% methanol in filtered sea water. Formol was not used since acidification of the medium could destroy the calcareous structures of the shell, which are indispensible for identification. Also, buffering with formol presents the drawback of polymerization of the aldehydes in a non-acid medium (Fraga, personal communication).

Preparation and study of the samples

The processes followed from the time the samples were taken until they were studied are set out schematically in Figure 3; a stereoscopic microscope were used for counting and a micropipette was used to remove the larvae from the sample. Since identification is preferably based on shell structure, mainly the hinge system, the first step consisted of cleaning and removing organic material using sodium hypochlorite. A 50% solution of commercial lye was used for approximately 20 minutes. If the valve did not separate spontaneously, the process was hastened using a needle set in a handle.

This first stage is the same for both optical microscope and SEM observations, and since the methodology for optical microscopic study has been described in detail by Pascual (1972) and Perez-Camacho and Roman (1973), among others, we will say only that the use of polyvinyl pyrrilidone as a mounting medium had drawbacks due to its acid pH which causes the valves to corrode. Neutralization leads to a loss of transparency which makes accurate observation difficult and

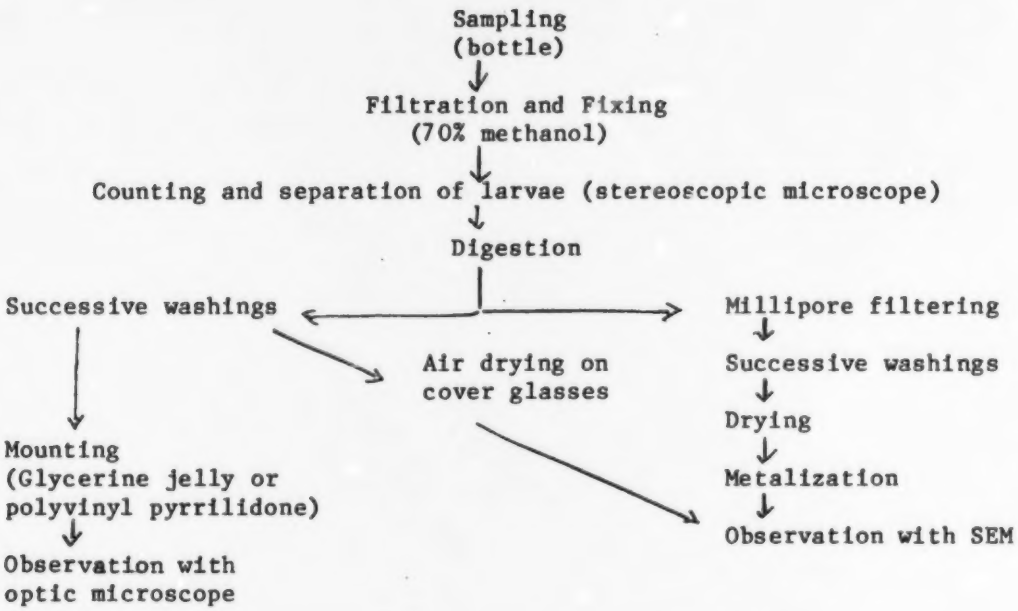

Fig. 3. Diagram showing the methodology followed in sampling, mounting, and observing lamellibranch larvae.

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therefore in this case glycerine jelly was preferred as the mounting medium, even though the process is more laborious.

For a SEM study of the samples, the valves must be completely clean and therefore successive washing with distilled water are necessary to avoid the appearance of precipitates when the samples dry.

Three washing methods were used:

a) Successive rinsings with distilled water using a micropipette.

This is very laborious on account of having to work on the stereoscopic microscope; there is considerable loss of larvae, but since the valves can be mounted on a cover glass, the background is uniform, clean, with almost no charge.

b) Washing in 47 μm mesh-size nylon sieves.

Shortens the washing time, although there is also a considerable loss of larvae, which may stick to the mesh. They can also be mounted on cover glasses, with the aforementioned advantages.

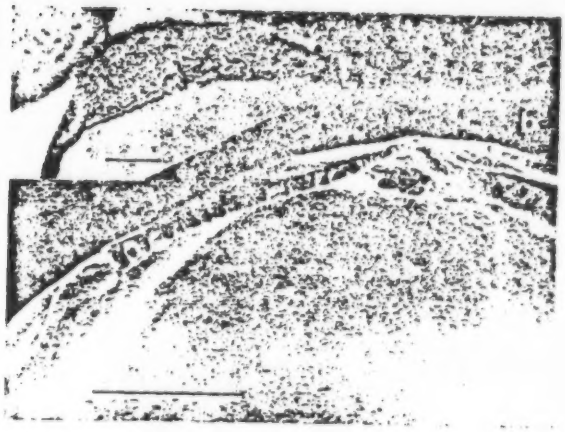

Fig. 4. Comparison of the results obtained using an optical microscope (mounting in glycerine jelly) (a), and SEM (b), in observing the morphology of the hinge of a Venerupis sp larva. The lines correspond to 30 μm.

c) Filtration and washing with millipore filters.

This is the quickest process and ensures almost total recovery of the specimens. The filter is the final mounting base; prior to final drying the valves can be arranged conveniently and a whole sample can be studied in a single mounting. However, the ground is often irregular and charges occasionally appear. Once dry, the filters were fixed in a sample holder (Stub), using a colloidal silver adhesive, and metallized in a Diode Sputtering POLARON 500 E with a SEM Stereoscan 180, Cambridge Instruments.

The results obtained using a microscopic optical microscope and a SEM can be compared in Figure 4.

RESULTS

We followed the studies by Rees (1950), Lutz and Hidu (1979), and Le Pennec (1980) in identifying the lamellibranch larvae. The same terminology has been used to describe them. Out of the total samples

Order FILIBRANCHIA
Suborder ANYSOMYARIA

Superfamily MYTILOIDEA
Family MYTILIDAE
Subfamily Mytilinae
Génus *Mytilus*
Mytilus edulis L., 1758

Superfamily PECTINOIDEA
Family PECTINIDAE

Superfamily ANOMIOIDEA *
Family ANOMIIDAE
Génus *Anomia*
Anomia ephippium (L., 1758)

Superfamily OSTREOIDEA *
Family OSTREIDAE
Génus *Ostrea*
Ostrea edulis L., 1758

Order EULAMELLIBRANCHIA
Suborder HETERODONTA

Superfamily CARDIOIDEA
Family CARDIIDAE
Subfamily CERASTODERMATINAE
Génus *Cerastoderma*
Cerastoderma edule (L., 1767)

Superfamily VENEROIDEA
Family VENERIDAE
Subfamily VENERINAE
Génus *Venus* L., 1758
Family PAPHIIDAE
Génus *Venerupis* Lmk, 1818

Superfamily TELLINOIDEA
Family TELLINIDAE

Suborder ADAPEDONTA

Superfamily SOLENOIDAE
Family SOLENIDAE

* Las larvas correspondientes a las familias Ostreidae y Anomiidae se han consignado juntas al ser imposible distinguirlas entre sí.

* The larvae of the Ostreidae and Anomiidae families have been classified together since it was impossible to distinguish between them.

for the annual cycle (March 1980 to March 1981) 8 taxonomic groups were distinguished, which have been classified following the criteria of Nordsieck (1969).

Mytilus edulis. In Mytilidae family species, the larval hinge develops very quickly. It occupies two-thirds of the dorsal edge of the shell and is formed by an uninterrupted series of teeth, whose number increases as the larva grows (Le Pennec, 1980; Lutz and Hidu, 1979). From the morphological viewpoint, three groups of teeth can be distinguished: the central ones which are small, and the anterior and posterior, which are wider (Fig. 5a). Although the morphology of the hinge is very characteristic in the Mytilidae family, it is difficult to identify the species. The form found has been assigned to M. edulis on account of its extraordinary abundance in the Estuary.

Pectinidae Family. In the larvae in D, the primitive cardinal platform presents a narrow central region, and teeth develop in the bases of the anterior and posterior oval depressions, generally three in number. Although extra teeth may appear after metamorphosis, the hinge of the larva consists of three or four anterior teeth and a similar number of posterior ones arranged symetrically at each end of a narrow central zone where the ligament is formed. Following the keys given by Le Pennec (1980) for various species of the family, all the larvae we observed correspond to Clamys opercularis, on account of the few teeth in the central part of the hinge (Fig. 5b), although we found some doubtful characteristics.

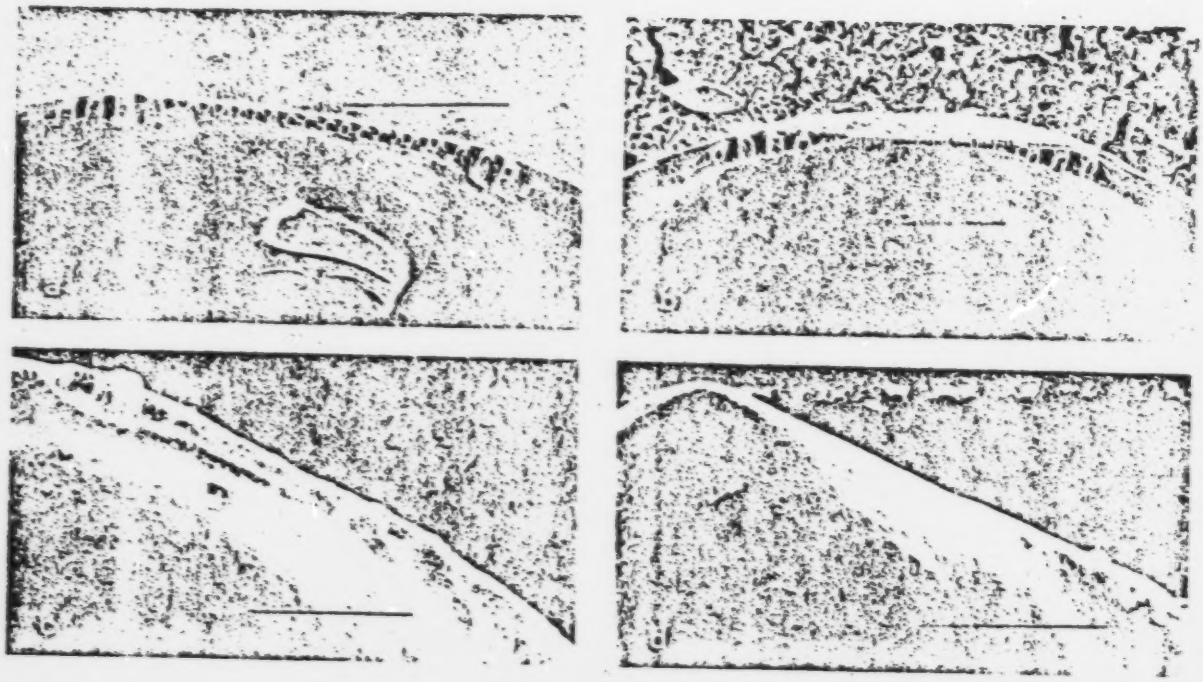

Fig. 5. Hinge structures of the lamellibranch taxonomic groups identified in plankton from the Pontevedra Estuary: a) Mytilus edulis (200 x 180 µm); b) Pectinidae family (180 x 150 µm); c) Ostrea-Anomia group (190 x 170 µm); d) Cerastoderma edule (90 x 70 µm). The lines correspond to 30 µm.

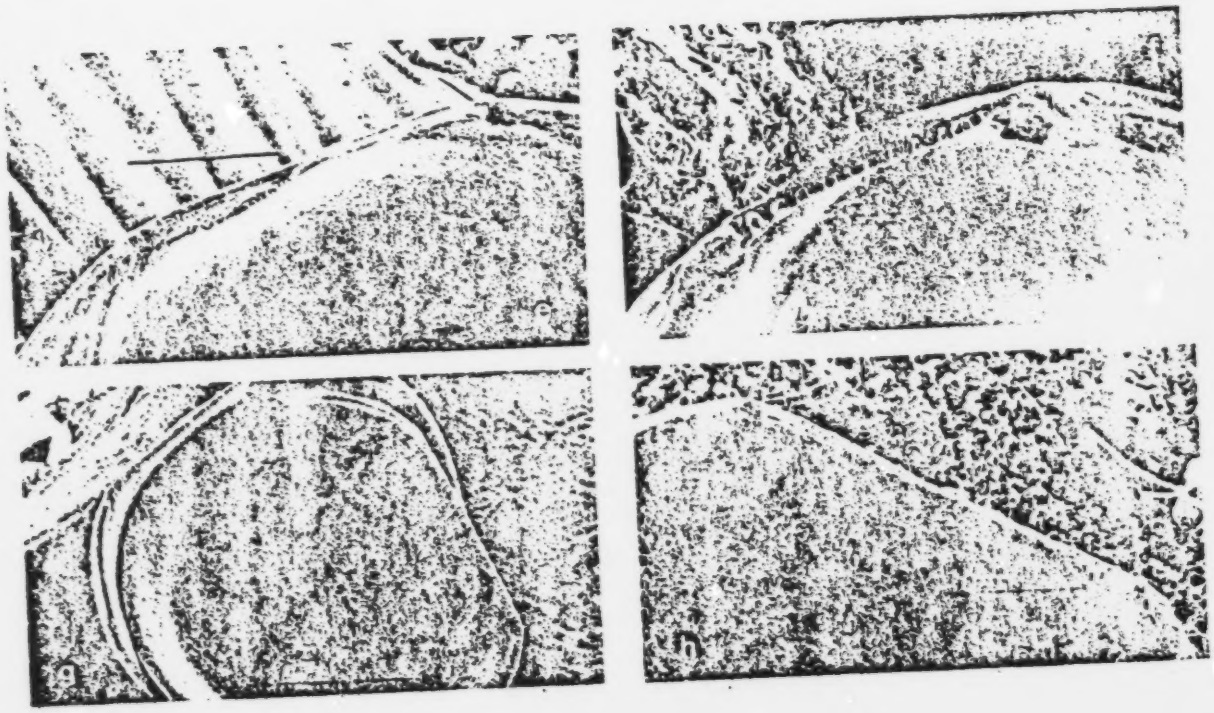

Fig. 6. Continuation of Fig. 5: e) Venus genus (240 x 210 μm); f) Venerupis genus (250 x 220 μm); g) Tellinidae family (75 x 65 μm); h) Solenidae family (180 x 125 μm).

Ostreidae-Anomiidae group. The similarity between the hinges of larvae of the Anomiidae and Ostreidae families, once they have reached 150 μm in length, has made it difficult to distinguish between the families since we did not find smaller-sized examples. According to Le Pennec (1980), once larvae of the Anomiidae family have reached 80 μm in length, a central block of approximately 20 teeth can be observed, with two larger anterior ones and another two [larger] posterior ones, plus an additional tooth in the left valve. The teeth are lost when the larvae reach a length of 300 μm. Ostrea edulis larvae (the most abundant species of Ostreidae in the Estuary) measure approximately 130 μm in length upon liberation [sic]. There are two teeth at each end of the hinge on the right valve, and one at each end of the left. After they reach 200 μm in length, which is when the teeth reach their maximum development, they begin to atrophy until they disappear when the larvae reach 300 μm (as in the Anomiidae family).

Due to the difficulties in distinguishing between the families, the larvae found in the plankton were compared with cultured Ostrea edulis larvae, and there were discrepancies with the description given by Le Pennec (1980). In O. edulis larvae from 200 to 350 μm, there is much variability in the number of teeth at the anterior and posterior ends of the hinge, ranging from examples with two teeth at each end to individuals with only one tooth in the entire hinge. In any event, we have preferred to include these larvae in a single group, Anomiidae-Ostreidae (Fig. 5c).

Cerastoderma edule. In the Cardiidae family the provinculum develops late. Figure 5d shows the hinge of a larva in D, before the hinge fold

has developed, which occupies one-half of the dorsal edge, is toothless, and disappears when the larva reaches 160 µm in length (Le Pennec, 1980). The sole tooth forms at approximately 170 µm, in the form of a rounded crest.

Venerupis sp. In this genus, the hinge system is formed by teeth of unequal sizes and in varying numers, occupying three-quarters of the dorsal edge. The smallest teeth are found at the anterior end (Fig. 6f). There are no descriptions available which make it possible for us to clearly distinguish the species of Venerupis to which the larvae found in the plankton in the Pontevedra Estuary belong.

Venus sp. In the Venus genus, the provinculum has irregular folds, occasionally with an isolated tooth. The fold is wedge-shaped with the narrow part ending in a point close to the ligament hollow (Fig. 6e). It was not possible to be certain in this case either of the species to which the larvae observed belong.

Tellinidae Family. According to Rees (1950), in this family the provinculum is formed by an uninterrupted series of miniscule, rectangular teeth (Fig. 6g); the ligament is displaced from the centre of the dorsal edge, and eyelash-type teeth appear on the two valves once the larva has reached 250 µm in length.

Solenidae Family. The larvae of species belonging to this family (Rees, 1950) present a single central tooth in the provinculum of the left valve, which fills it almost completely, and a hollow in the anterior

and posterior ends (Fig. 6h). The right valve, however, has a tooth at each end, which corresponds to the hollows in the opposite valve. Existing descriptions do not make it possible to distinguish the species in this case either.

CONCLUSIONS

The use of SEM gives better results than optical microscopes, not only from the viewpoint of better resolution and tridimensional view of the object (Le Pennec, 1980), with the possibility of clearly differentiating between teeth and hollows in the hinge system and the ligament hollows (Fig. 4), but also because it makes it possible to mount a large number of individuals per sample, which is practically impossible with an optical microscope because of the rapid solidification of the mounting media normally used.

However, to obtain optimum results from the viewpoint of identification of lamellibranch planktonic larvae it is necessary to improve their descriptions, based on cultured examples covering all stages of development - which is perhaps the only way of solving the problem of doubtful classifications.

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